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Seedling Transplant Selection Does Not Cause Genetic Shifts in Genebank Populations of Inbred Potato Species

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ABSTRACT

A basic goal of the U.S. Potato Genebank (USPG) and others is to test assumptions about the stability of genetic diversity in their collections. For example, when heterogeneous seed populations are regenerated, one assumes that using a careful regeneration protocol will result in very little diversity loss in the progeny. However, even the most careful mating scheme cannot prevent genetic selection if it happens earlier—when seedlings are transplanted to become the seed-increase parents. The objective of this work was to assess the prospect of losing diversity at the seedling transplant step. Seeds of a total of 245 original seedlots (from the wild) of 11 inbreeding species [*Solanum acaule* Bitter, *albicans* (Ochoa) Ochoa, *demissum* Lindl., *etuberosum* Lindl., *fendleri* A. Gray, *polyadenium* Greenm., *palustre* Poepp. ex Schltdl., *polytrichon* Rydb., *papita* Rydb., *stoloniferum* Schltdl. & Bouché, and *verrucosum* Schltdl.] were sown. The most vigorous, uniform "normal" (N) seedlings were distinguished from any that were small (S) or otherwise would likely be avoided when transplanting the parent plants to be used for seed increase. Bulks of N and S types within seedlots were compared by random amplified polymorphic DNA (RAPD). Genetic similarity (GS) was calculated as average percentage matching band status at RAPD loci. About 25% of the seedlots exhibited S types, but in no case were these significantly different from their N sibs (none with lower than GS = 98%). These results suggest that inbred species' original seedlots are homogeneous and not subject to unwanted seedling selection at transplanting, despite random environmental effects that sometimes produce S seedlings.

The GREEN REVOLUTION started the process of replacing in situ crop diversity with genetically uniform cultivars tailored to maximize production under ideal conditions, often within a system of high-input agriculture. Concern over the resulting loss of crop genetic diversity motivated the creation of ex situ genebanks to preserve it. Thus, the agricultural strategy in some locations changed from genetic diversity in space with stability over time, to genetic uniformity in space (e.g., perhaps even the extreme of widespread monocultures at any given time). This situation focuses a great responsibility on genebanks to store germplasm of high quality and rapid availability so breeders will have the genetic tools to generate diversity over time, as needed to meet changes in growing conditions, diseases and pest pressures, consumer preferences, etc. Genebanks become increasingly important as in situ landraces are eliminated and wild relatives are lost because of habitat degradation.

Assuming that the genebank is able to keep at least some living propagules in distributable form for each of its accessions, the next concern is to prevent unfavorable genetic shifts that could occur within populations especially when seed multiplication is performed. There are various ways that such genetic shifts could take place.

Theoretical population genetics models have rarely been applied to the practical problems of maintaining captive populations in genebanks (Brown and Marshall, 1995; Crossa and Vencovsky, 1999; Sackville Hamilton et al., 2003). Factors affecting vulnerability of alleles to loss when populations are multiplied in genebanks include the number of parents used, the mating and seed bulking scheme that determines the effective parental population size (N_e), the frequency of the hypothetical allele to be preserved, the number of alleles per locus, and the number of such loci (Bamberg and del Rio, 2003). However, meticulous care in handling seed increase parents may come too late to be effective if much of the original diversity is lost earlier—by inappropriate collecting, preferential germination, or inadvertently excluding certain genotypes when choosing among excess seedlings at transplanting.

Gale and Lawrence (1984, p. 79) remark, "To allow for seedling mortality, it will presumably be necessary to sow more than one (or two) seeds per parent. We stress the need to avoid natural selection when thinning seedlings to one (or two) per parent; the seedling(s) to be retained must be chosen at random from those available."

But use of genetically random (i.e., representative) individuals as seed increase parents cannot be taken for granted. Excess seeds are always sown to make sure enough germinate and produce seedlings available for use as parents. Variation in physiological age, effects of the particular seed-processing or sprouting micro-environment, or the presence of diseases among individual seeds could cause seedlings to appear non-uniform at transplanting for non-genetic reasons. In that case it would be counterproductive to include abnormal seedlings suspected of having a lower potential for rapid growth, profuse flowering and fertility. On the other hand, if abnormal seedlings are genetically different, it may be worthwhile to intentionally include them to preserve the genetic diversity of the population. We say "may" because genetic differences could be caused by deleterious mutants (Schoen et al., 1998) that require a

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Abbreviations: GS = genetic similarity, the simple matching coefficient, or percentage RAPD loci with matching band status; USPG = U.S. Potato Genebank, NRSP-6.

disproportionately high level of genebank resources for their maintenance, considering their poor prospects for practical agricultural exploitation.

Previous work of the authors concluded that there is little genetic difference between seed generations (del Rio et al., 1997), or between clonal collections from the wild and their genebank-generated seed progeny (del Rio and Bamberg, 2003), suggesting that selection at transplanting (or otherwise) does not significantly erode genetic diversity. However, systematic evaluation of the potential effects of seedling selection on a wide array of potato germplasm had not been done, so became the objective of this study.

MATERIALS AND METHODS

Test materials were produced at USPG and RAPD bands were generated at the University of Wisconsin, Madison, Department of Horticulture. Materials were selected on the basis of the following rationale: Most potato species are outcrossing diploids expected to produce segregating progeny, especially when seedlots originate from deliberate hand intermating in the genebank. Such seedlots would be expected to segregate S types, but exclusion of such S types would likely result in the loss of only a few particular alleles that confer the S phenotype and would not be consistently linked with a large number of other unique alleles and a *general* loss of diversity. In contrast, the most serious threat of losing allelic diversity at many loci at once would occur in accessions of inbreeding species potentially composed of a mixture of homozygous individuals. The deselection of certain homozygotes in such seedlots could cause large genetic shifts, resulting in complete loss of significant amounts of genetic diversity from the seedlots (e.g., Roos, 1984; Tao et al., 1992).

One problem in testing this is that USPG-generated inbred seedlots might not exhibit S seedlings because they had already been eliminated when transplanting for previous seed increases. Therefore, we selected a set of materials consisting of all available *original* seedlots of inbreeding species. *Original* as used here means the seeds tested were those collected directly from the wild and not progeny generated from seed increase done at the genebank. The materials consisted of 245 original seed populations of 11 different species (*Solanum acaule*, *albicans*, *demissum*, *etuberosum*, *fendleri*, *polyadenium*, *palustre*, *polytrichon*, *papita*, *stoloniferum*, and *verrucosum*).

Two replicates of 50 seeds were planted for each population. The standard method of seedling rearing was used. Seeds were soaked in 2 g L^{-1} GA_3 for 24 h, rinsed with tap water, and washed onto the surface of packed soilless potting mix in 8 cm clay pots, then covered with about 3 mm of fine puffed mica (vermiculite). Pots were kept in the greenhouse at about 25/20°C (d/n), 16-h days, and watered daily. At 3 wk post-sowing, seedlings were at the usual size for transplanting: about 5 cm tall with two true leaves. They were assessed for presence of small or otherwise unthrifty individuals that probably would not be selected for transplanting. The S and the remaining N seedlings from each population were transplanted into 6-cm peat pots in 54-unit plastic flats. Bulk DNA samples were made by pooling equal amounts of leaf tissue from seedlings within each class from each seedlot within each replicate. Since S seedlings were, by definition, the unusual minority types, all available S seedlings were bulked for a DNA sample. In contrast, the number of N seedlings was expected to be at least 27, which conveniently fit into half of the standard transplanting flats, so a maximum of only 27 N seedlings were bulked, even when more were present. Previous work had shown that this number of individuals in a bulk results in a very stable RAPD banding pattern, even for very heterogeneous populations (Bamberg and del Rio, 2004). The RAPD bands were generated by the method described in del Rio et al. (1997). An average of 210 RAPD loci were generated to compare the S and N classes.

The analysis was designed to determine the proportion of seedlots that produced S seedlings and to assess their genetic distinction from N seedlings in the same seedlot. The RAPD results for N and S seedlings were compared by calculating GS, the percentage of RAPD loci with matching band status. The limited size of S bulks increased the risk of detecting false N:S differences for relatively low-frequency bands actually present in both N and S seedlings. This is because the band allele would be more likely to be missing from a small bulk, by chance, than from a large one. For this reason, the GS calculation was made more conservative by basing it only on N:S dissimilarities in which the S bulk had a band absent in the N bulk.

Statistical significance of GS was determined as follows. The GS of all replicates, (i.e., N:N and S:S within seedlots) were calculated. The average of these was hypothesized to represent the random error resulting from all aspects of RAPD band generation and scoring. The distribution of individual replicate GS was then assessed by a chi-square test for fit to that expected from a binomial distribution, with p equal to the observed mean replicate GS. Individual GS of N:S comparisons within populations were assessed for significance by calculating the probability that a GS as low as observed would occur in a single sample from a binomial distribution, with p equal to that of the observed mean replicate GS. Finally, it was necessary to adjust this probability to reflect a conclusion that no individual GS as low as the one observed would be likely to occur in a sample size of as many N:S comparisons as were actually observed in the experiment. The critical level for declaration of statistical significance was set to correspond to a GS low enough to have a $p \leq 0.05$, by chance according to sampling from the binomial distribution (Bamberg et al., 2001).

RESULTS

No S seedlings were observed for 178 of the 245 comparisons (about 75%). This proportion appeared to be typical regardless of species (Table 1). The seedlots with S seedlings and for which RAPD comparisons were made had a total germination rate of about 66%, or about 34 total seedlings per 50-seed germination unit. On average, about 1/5 of these (7) were S seedlings, and the remaining (27) seedlings were of the N type.

Sufficient RAPD data was obtained for 54 of the seedlots exhibiting S seedlings. The average GS of S:S and N:N comparisons (i.e., among replicates) was very high, both being 99.8%. The chi-square tests determined that the observed distribution of both individual S:S and N:N GS values were likely outcomes from a random sample from a binomial distribution with $p = 0.998$ (chi-square $p = 0.75$ for S:S and 0.21 for N:N). Thus, the generation of RAPD fingerprints was shown to be highly and equally consistent across all materials, as is consistent with our previous reports (del Rio and Bamberg, 2000). The GS of N:S comparisons ranged from 100% (36 of the comparisons) to 98.4% (Table 1). The probability of observing GS = 98.4% between two random samples pulled from the same population at

Table 1. Occurrence of small seedlings (S) by species and their genetic similarity (GS) to normal (N) seedlings from the same seedlot.

Number of seedlots	*Solanum* species†											
	acaule	*albicans*	*demissum*	*etuberosum*	*fendleri*	*polyadenium*	*palustre*	*polytrichon*	*papita*	*stoloniferum*	*verrucosum*	Total
Total	**110**	**2**	**16**	**21**	**18**	**1**	**47**	**10**	**3**	**15**	**2**	**245**
No S observed	**81**	**1**	**13**	**12**	**14**	**1**	**32**	**9**	**3**	**11**	**1**	**178**
S observed. . .	**29**	**1**	**3**	**9**	**4**	**0**	**15**	**1**	**0**	**4**	**1**	**67**
. . .but insufficient DNA for RAPDs†	**5**	**0**	**1**	**1**	**0**	**0**	**6**	**0**	**0**	**0**	**0**	**13**
. . .and RAPDs generated	**24**	**1**	**2**	**8**	**4**	**0**	**9**	**1**	**0**	**4**	**1**	**54**
GS of N:S based on RAPDs‡	**0.987–1.00**	**1.00**	**0.989–1.00**	**0.984–1.00**	**0.995–1.00**	**NA**	**0.991–1.00**	**0.995**	**NA**	**1.00**	**1.00**	

† Only one rep had S, so no S:S RAPD comparison was possible, or S seedlings were not vigorous enough to provide DNA. In the former case, there were never more than three S in the other rep.

‡ Range of observations. No observed GS of S:N is significantly lower than the observed GS within seedling types (S:S and N:N) of 0.998. Calculations based on an average of 210 RAPD loci.

least once in 54 trials is slightly greater than $p = 0.05$, so we conclude that GS between N:S pairs as low as those observed in the experiment are all adequately explained by chance.

DISCUSSION

We have long wondered whether populations of inbreeding species in the genebank are homogeneous (i.e., a single genotype). The question is of great importance from a conservation standpoint, since the genetic diversity of heterogeneous inbreds is the most vulnerable to loss by inadvertent selection, while genetic diversity of homogeneous inbreds is the least vulnerable. As mentioned previously, for heterogeneous inbreds, selection happening just once and on the basis of just one allele could concomitantly eliminate many other linked alleles. But, in contrast, homogeneous inbreds are simply one parental genotype capable of producing only one identical progeny genotype. So, discounting mutations, complete conservation is assured. Recent evidence on a limited number of populations and species has suggested that plants within populations of inbred *Solanum* species are highly homogeneous (Bamberg and del Rio, 2004). But that evidence came from genebank-generated seedlots that might already have been subjected to selection during the seed increase process.

What happens at collection must also be considered. At least one case is known in which two distinct inbreds occur at the same collection site (evidenced by tuber collections), but only one of these types was collected as seed, perhaps because only one type was fruiting at the time (Moreyra-P. et al., 2004). And, with respect to individual alleles, at least one case has been documented in which a useful recessive trait was linked to the S phenotype in a collection of mixed inbreds. A total of only 22 seedlings were available, so all were transplanted. Otherwise, the S type, along with the unique mutant, probably would have been discarded (Fernandez and Bamberg, 2005).

While mixed inbred populations should logically have a greater risk of significant genetic shifts because of seedling selection, most wild potato species are outcrossers. The authors are currently retesting observations that seedling selection can have significant genetic impact on some outcrossing populations (Bamberg et al., 2003).

This experiment did not address risk of losing diversity if certain types are less likely to germinate. This is certainly a possibility whenever germination is less than 100%. Obviously, the expectation of <100% germination is why the genebank manager sows excess seeds. Unlike the potential loss of diversity by deselection of S seedlings, however, loss of diversity in ungerminated seeds cannot be easily avoided if the presumed optimal germination protocols are already being followed.

This experiment suggests that even original seedlots of inbred species are already homogeneous. That conclusion assumes that mixed inbred genotypes would be distinguished as S and N seedlings and that the genetic difference between S and N genotypes involved enough RAPD alleles to be detected (this experiment resolved populations when only about 2% difference in RAPD alleles was measured). Even if these populations were not homogeneous, the results of this experiment indicate that, in practice, no *general* loss of diversity is likely to be associated with transplanting only the N seedlings. This conclusion is strengthened when one remembers that concerns about selection are moot for most (75%) of the seedlots, since they did not produce any S seedlings.

With appropriate sampling, RAPDs reliably resolve small genetic differences in potato (del Rio and Bamberg, 2000) or in this case, verify a lack of such differences. While interspecific and interpopulation GS were not the focus of this study, the RAPD bands analyzed in that way are useful as a positive control. Thus, 100% of species and 80% of populations within species were significantly distinguishable, having much lower GS than any N–S comparisons observed within populations.

For potato, and probably most other crops, much more diversity exists than can be preserved in genebanks with the current available funds. So maximizing the impact of genebanks depends on efficiency, both in the form of adopting better methods and eliminating protocols that are unnecessary or even counterproductive. Here we determined that there is no reason to make an extra effort to include S seedlings of inbreeding species, especially if their reduced vigor as seedlings is associated with lower survival rate, slower growth, and reduced fertility and seedset.

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